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# Ultrastructural Differences Between Longitudinal and Circular Muscle Cells of the Guinea Pig Stomach

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The ultrastructure of the longitudinal and circular muscle cells of the guinea pig stomach, known to display different contractile responses, was compared. The longitudinal muscle layer consisted of about 20 layers of smooth muscle cells and the extracellular space occupied about 12.1% of the cross sectional area. The circular muscle layer consisted of closely packed muscle bundles arranged side by side. The extracellular space within the bundle represented about 4.4% of the cross sectional area. Nexuses were consistently found in the circular muscle layer but could not be found in the longitudinal muscle layer. Numbers of both mitochondria and microtubules per unit area of smooth muscle cell were larger in the longitudinal than in the circular muscle. The cell area occupied by the sarcoplasmic reticulum was about 4.7% in the longitudinal muscle cell, twice as much as in the circular muscle cell (2.3%). Numbers of caveolae per micrometer of the cell perimeter were almost the same in both tissues. There were approximately 25 and 50 thick filaments per 0.5 $\mu m^2$ of cytoplasmic area in the longitudinal and circular muscle cell, respectively. A lower pH fixative (cacodylate, pH 6.6) gave a better contrast of specimens than other fixatives used, and an organic buffered (PIPES) fixative led to a more regular arrangement of myofilaments. But the characteristic distribution of the thick filaments between both muscles was not different among the specimens fixed with these fixatives.

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## Introduction

Longitudinal and circular smooth muscles of the guinea pig stomach are known to show particular physiological and pharmacological characteristics with respect to contractility. In response to K-depolarizing solution, contraction of the longitudinal muscle strip involves a short phasic phase followed by a longer tonic phase, whereas the circular muscle strip shows only a transient contraction[1]. They respond differently to prostaglandin[2~4], and to Na-free solution and electrical stimulation[1].

It is generally accepted that the contractile mechanism of smooth muscle is basically the same as that of striated muscle, i. e. thick and thin filaments work together as a force-generating apparatus[5,6]. However, smooth muscles show different contractility from tissue to tissue, as described above. There may be many other factors that affect the contractile process. The tissue specificities may be accompanied by structural differences in the smooth muscle cells. Devine *et al.*[7] estimated the volumes of the sarcoplasmic reticulum of several smooth muscle tissues and found that the volume was greater in the tonic smooth muscles than in the phasic (spike generating) smooth muscles.

The purpose of this work is to compare as quantitatively as possible, the fine structure

of smooth muscle cells in longitudinal and circular muscles of the guinea pig stomach, which are distinctly different in their contractility properties. A brief description of some of the results reported here has been presented before as a short communication[8].

## Materials and Methods

Nine guinea pigs weighing between 300 and 450 grams were used for electron microscopy. They were stunned and bled. The stomach was excised and dissected along the greater curvature. Longitudinal muscle strips (1.5–2 mm wide, 15–20 mm long) were prepared by dissecting along the fringe in the region of the corpus and separating the strip from the mucosal layer. The circular muscle strips were dissected out perpendicular to the longitudinal muscle preparation from the same region.

Each strip was mounted in an organ bath and attached to a kymograph. After 30 min or more incubation in a physiological solution (mM: NaCl, 125; KCl, 5.7; $CaCl_2$, 2.0; $MgCl_2$, 0.5; $NaHCO_3$, 15; glucose, 12) at 37°C, aerated with 95% $O_2$ and 5% $CO_2$, the muscle was relaxed by adding adrenaline (final concentration; $10^{-5}$ M), and then pre-fixed by replacing the solution with a warm fixative (see below). The length change of the muscle strips during fixation was negligible. The fixed tissue was detached from the mounting lever of the apparatus and cut into small pieces in a fresh fixative. Some stomachs were fixed immediately after removal from the body at room temperature, and the corpus part was dissected after a few minutes, and cut into small blocks in fresh fixative.

The tissues were pre-fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at room temperature for 2 hr, washed with 0.1 M cacodylate buffer containing 0.2 M sucrose for more than 1 hr and then post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer containing 0.2 M sucrose at low temperature (0～4°C) for 1 hr.

To ensure optimal preservation of thick filaments the following buffer solutions were used, based on the findings of previous investigations[9~11]: (1) 0.1 M cacodylate buffer, pH 6.6, (2) 0.1 M cocodylate buffer, pH 6.6 plus 10 mM $MgCl_2$, and (3) 0.1 M piperazine N-N′-bis [2 ethanol sulfonic acid (PIPES)], pH 7.6.

The specimens were stained en bloc with 2% uranyl acetate for 1 hr, dehydrated in an ethanol series, and embedded in Epon 812. Thin sections cut transversely to the longitudinal axis of muscle cells with glass knives, were stained with 0.2% lead citrate[12].

### *Analytical Study*

Profiles of transversely sectioned cells at the level of the nucleus or at the level of the polar regions of the nucleus where organelles are concentrated were not selected for analysis. The area and the perimeter of each cross-sectioned cell (magnified to ×30,000) were estimated by using a film analysing system (Model 514, Oosawa Sho-Kai, Tokyo). The portions of the sarcoplasmic reticulum (SR) were carefully painted with black ink and then all of the painted areas were integrated by a Photo Pattern Analyzer (Model 250, Applied Electric Lab. Co. Ltd., Japan). The SR content was expressed as a percentage of the cross-sectional area of each smooth muscle cell.

The other cell organelles, mitochondria, microtubules and caveolae were counted on each profile and were expressed as numbers per unit cross sectional area or per unit-length of

the perimeter of the cell.

The extracellular space within the muscle bundles was also estimated by the Photo Pattern Analyzer by subtracting the area of painted cells from a selected area of 300 $\mu m^2$ in a picture magnified to $\times 9{,}000$.

## Results

*Characteristics of Potassium Contractures of Longitudinal and Circular Muscles*

In response to K-depolarizing solution (Na in normal solution was replaced by K), the longitudinal muscle strip contracted gradually and reached the maximum shortening level after 10～15 min. In some cases, it showed a shoulder peak at the beginning of isotonic contraction. On the other hand, the circular muscle strip contracted quickly to its maximum level but then relaxed within a minute almost to the initial level. Although the time required to reach the maximum shortening level was greatly different between the two muscles, the magnitude of contraction was almost the same (about 50% of resting length at load around 10% of the force generated by the tissue). These contractile patterns correspond well to those seen by Kuriyama *et al.*[1] who measured isometric contractions.

*Tissue Construction*

The muscle layers of the guinea pig stomach in the corpus region were around 0.2 mm thick, estimated from thick sections of Epon embedded tissue by light microscopy (the value was not corrected for the shrinkage of tissue during fixation and embedding). The outer, longitudinal muscle layer, occupied one third of the thickness, and consisted of about 20 layers of muscle cells. In the inner, circular layer, which occupied two thirds of the thickness, muscle cells were densely packed in large bundles (approximately 0.02 $mm^2$ in cross-sectional area) arranged side by side.

Fig. 1 shows cross sectioned profiles of the longitudinal and circular muscle layers at low magnification. Although the strip had been fully relaxed with adrenaline ($10^{-5}$ M), longitudinal smooth muscle cells are irregular in shape (Fig. 1 A). The extracellular space between the longitudinal muscle cells was $12.1 \pm 1.0\%$ of the cross sectional area. On the other hand, the muscle bundle of the circular layer was densely packed with muscle cells which had a rather smooth surface (Fig. 1 B) and the extracellular space was approximately $4.4 \pm 0.6\%$ of the cross sectional area.

As shown in Figs. 1 B and 2 B nexuses were seen between some circular muscle cells, but could not be found in the longitudinal layer. Other cell to cell contacts, intermediate junctions and simple appositions[13], were seen in both layers, but more frequently between the circular muscle cells. While interdigitations between two muscle cells sometimes occurred in the longitudinal muscles, this was rarely observed in the circular muscles.

Nerve fibers were commonly observed among the muscle cells in both layers (Figs. 1 A and 2 B). The axons were grouped in varying numbers surrounded by Schwann cell process and ran parallel with smooth muscle cells. The varicosities observed in very rare cases in transverse sections, did not exhibit any specific differences in vesicular content between the longitudinal and the circular muscle layers. Small, round agranular vesicles, small flattened vesicles, and large granular vesicles were usually observed in such varicosities.

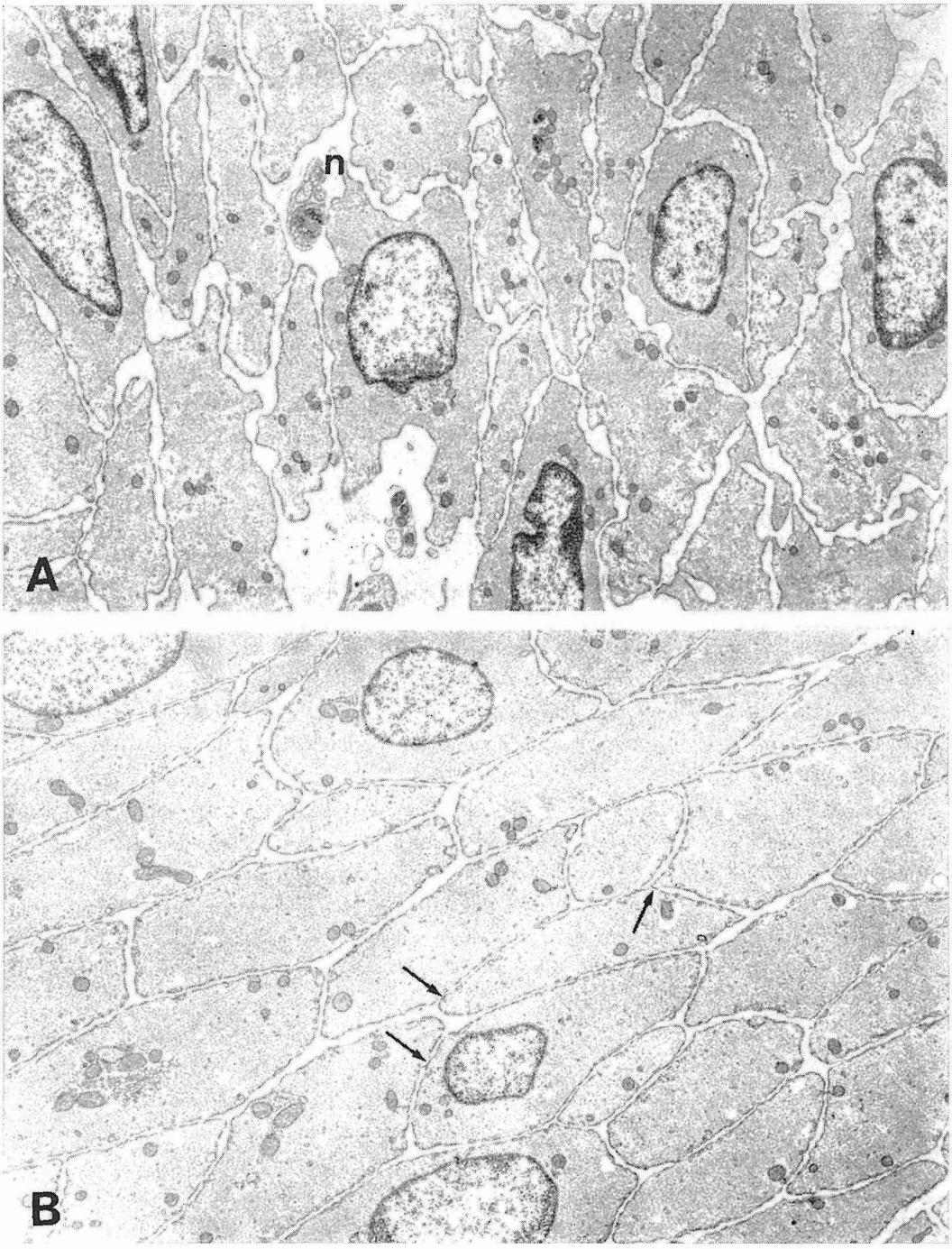


**Fig. 1** Low magnification view of transverse sections of guinea pig stomach muscle layers. *A* longitudinal layer; *B* circular layer. Note the irregularly shaped smooth muscle cells and wider extracellular space in *A*. A nerve bundle (n) is seen in *A*. Arrows in *B* show nexuses. Tissues relaxed with adrenaline, then fixed with glutaraldehyde buffered with 0.1 M cacodylate, pH 6.6 (Magn. ×7,000).

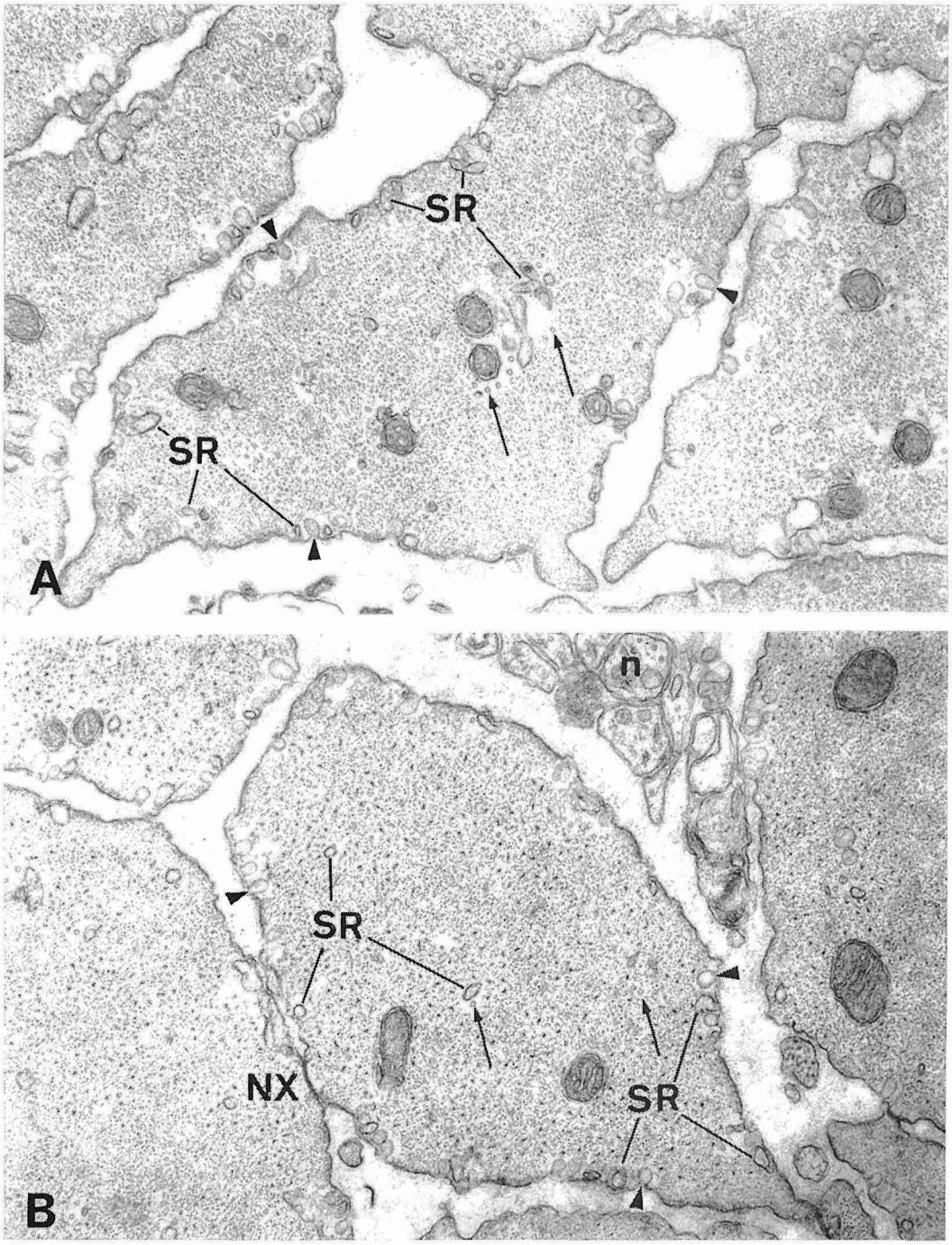


**Fig. 2** Transverse sections of smooth muscle cells in guinea pig stomach. *A* cells of longitudinal muscle layer; *B* cells of circular muscle layer. Sarcoplasmic reticula (SR) distribute adjacent to caveolae (arrowheads) and to mitochondria. Microtubules (arrows) run along mitochondria or SR. Thick filaments appear more distinctly in *B* than in *A*. A nexus (NX) can be seen in *B*. Nerve axons (n), bunbled by Schwann cell process are also seen in *B*. Fixed with cacodylate-buffered (pH 7.4) glutaraldehyde and osmium tetroxide, and block-stained with uranyl acetate (Magn. ×30,000).

*Intracellular Structure*

Smooth muscle cells in the longitudinal and circular layers are shown at higher magnification in Fig. 2. The largest part of each smooth muscle cell was occupied by myofilaments. The other cell organelles, mitochondria, SR and microtubules were dispersed among the myofilaments. These cell organelles seemed to be related to each other, since the mitochondria were always associated with SR or caveolae or both, and microtubules were located adjacent to the SR. The greatest number of SR elements was seen at the periphery of cells, where clusters of caveolae were formed (Fig. 2).

**Table 1** *Distributions of cell organelles in longitudinal and circular muscle cells of the guinea pig stomach* (corpus)

| | Animal | SR % distribution* | Mitochondria no./10 $\mu m^2$ | Microtubules no./$\mu m^2$ | Caveolae no./$\mu$m (perimeter) |
|---|---|---|---|---|---|
| 1+ | Long. (23) | 4.68±0.48 | 9.6 | 4.4±1.3 | 2.2±0.5 |
| | Circ. (25) | 1.98±0.41 | 5.3 | 2.1±0.7 | 1.9±0.7 |
| 2+ | Long. (21) | 3.72±0.88 | 8.5 | 5.3±1.7 | 2.0±0.4 |
| | Circ. (20) | 1.92±0.32 | 4.8 | 1.8±0.7 | 1.8±0.6 |
| 3+ | Long. (11) | 4.92±1.39 | 5.6 | 5.2±1.7 | 1.4±0.3 |
| | Circ. (12) | 2.48±0.67 | 5.7 | 1.7±0.2 | 1.9±0.3 |
| 4# | Long. (19) | 5.21±1.19 | 10.2 | — | 1.7±0.3 |
| | Circ. (21) | 2.76±0.62 | 5.3 | — | 2.2±0.5 |
| 5# | Long. (12) | 4.72±1.40 | 6.9 | — | 1.8±0.2 |
| | Circ. (18) | 2.08±0.33 | 3.5 | — | 2.1±0.3 |
| 6# | Long. (26) | 4.87±1.17 | 5.6 | — | 1.7±0.6 |
| | Circ. (24) | 2.30±0.70 | 3.3 | — | 1.5±0.5 |

Values are given as means±SD. Long.; longitudinal muscle cell, Circ.; circular muscle cell. The number of cells measured is given in parentheses. * percentage of cell cross sectional area (excluding nucleus) occupied by the sarcoplasmic reticulum (SR); +: fixed at relaxation; #: fixed at length *in situ*

The distribution of cell organelles in both types of smooth muscle cells is summarized in Table 1. The percentage area of SR in a cross section of longitudinal muscle cells averaged about 4.7, and was twice that of circular muscle cells (2.3%). The count of microtubules (characterized by a uniform circle about 250 Å in diameter) per $\mu m^2$ was also greater in the longitudinal than in the circular muscle cell. Mitochondria in smooth muscle cells were elongated along the long axis of the cell and were circular in transverse sections. The longitudinal muscle cell usually contained more mitochondria per unit cross-sectional area than the circular muscle cell. The densities of caveolae were almost the same in the two types of muscle.

From the results of Table 1, it was concluded that the longitudinal smooth muscle cell contained greater concentrations of every intracellular organelles except myofilaments.

*Myofilaments*

The most striking difference between the longitudinal and circular muscle cells was in the appearance of thick filaments. In spite of the application of exactly the same method

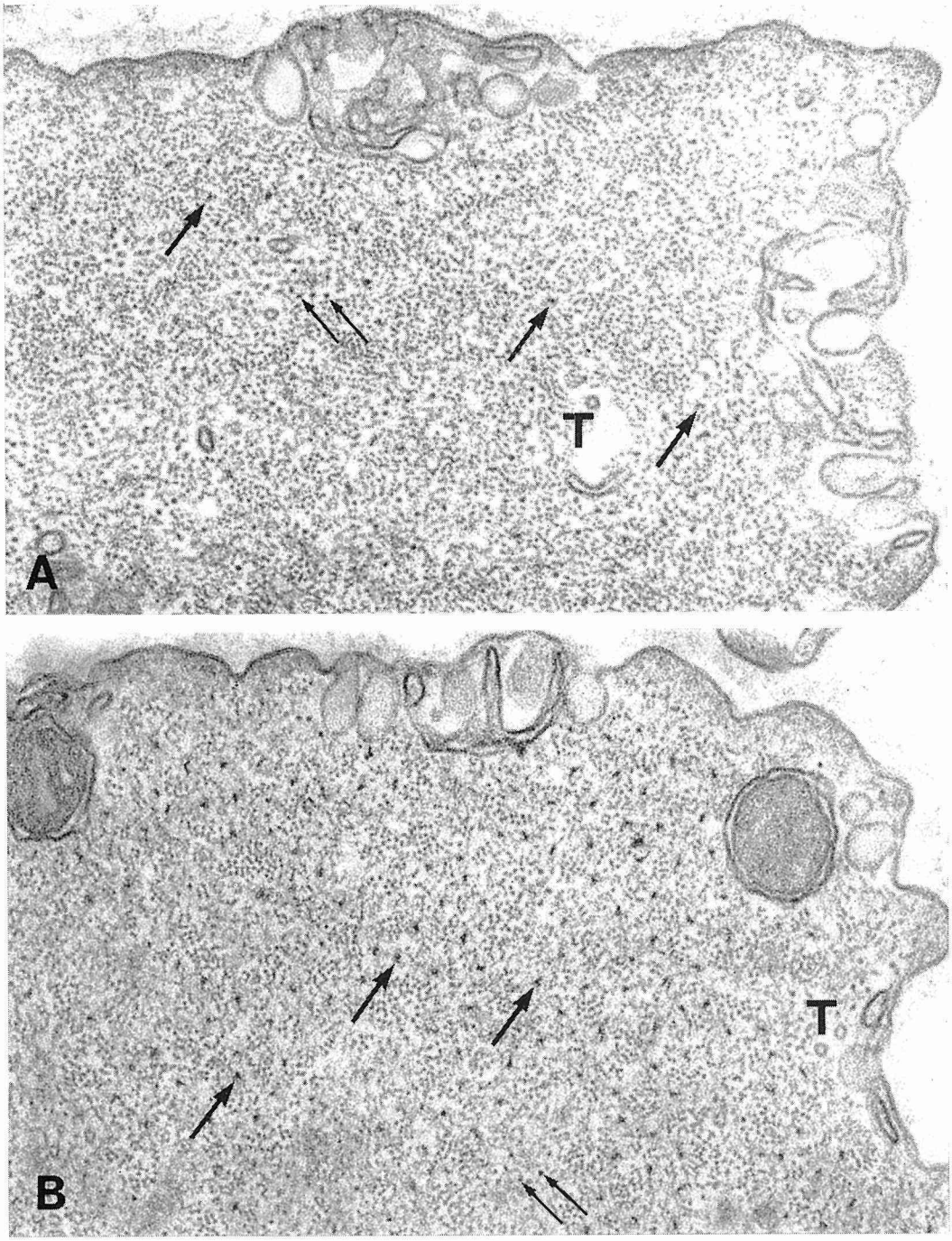


**Fig. 3** Transverse sections of parts of smooth muscle cells of guinea pig stomach. *A* longitudinal muscle cell; *B* circular muscle cell. The thick filaments (large arrows) in *B* are greater in number and size than the thick filaments in *A*. Intermediate filaments (small arrows) are present frequently at the periphery of the dense bodies. *T* microtubule. Fixed with glutaraldehyde and osmium tetroxide in PIPES buffer, pH 7.6, and block-stained with uranyl acetate (Magn. ×80,000).

of electron microscopy, thick filaments were not demonstrated as clearly in the longitudinal muscle cells as in the circular muscle cells (Fig. 2).

At higher magnification, the thick filaments were certainly distinguishable even in the longitudinal muscle cells, and set at the center of a round or oval electron lucent area surrounded by uncertain numbers of thin filaments (Fig. 3). However, thin sections cut perpendicular to the myofilaments showed that most of the thick filaments in the longitudinal muscle cell were much thinner than those in the circular muscle cell. Furthermore, the density of distribution of thick filaments was low in the longitudinal compared with the circular muscle cell (Table 2).

**Table 2** *Densities of thick filaments of smooth muscle cells in the guinea pig stomach* (corpus)

| Animal | Buffer solution | Counts per 0.5 $\mu m^2$ crossed area | |
|---|---|---|---|
| | | Long. | Circ. |
| 1 | Cacodylate, pH 7.4 | 25.3± 8.4 (12) | 45.8± 3.2 (12) |
| 2 | Cacodylate, pH 6.6+10 mM $MgCl_2$ | 28.9±10.8 (12) | 57.2± 5.0 (12) |
| 3 | PIPES, pH 7.6 | 24.5± 7.6 ( 6) | 46.4± 7.0 ( 8) |

Thick filaments were counted in 0.5 $\mu m^2$ cytoplasmic areas (excluding nucleus and mitochondria) on electron micrographs enlarged to ×100,000. Values are given as means±SD. Data are compared among animals fixed in different buffer solutions, as well as between longitudinal (Long.) and circular (Circ.) muscle cells. Numbers of experiments are in parentheses.

The use of a lower pH buffer solution (pH 6.6) resulted in a better contrast of specimens compared with those fixed at pH 7.4 (Fig. 1). Other characteristic features including thick filaments, however, seemed to be unchanged by the pH.

An organic based buffer, PIPES, gave almost the same structural features as cacodylate buffer. A small advantage of using the latter solution was that it seemed to result in a more regular arrangement of thin filaments than the former (Fig. 3).

Intermediate filaments (100 Å filaments) were usually observed surrounding the periphery of dense bodies in both types of smooth muscles. However, in some longitudinal muscle cells, they were greatly increased in number in the inner part of the cell.

There were a few smooth muscle cells different in structure from most other circular muscle cells within the circular muscle bundle. They were characterized by their irregular shape, high content of cell organelles and by their ambiguous thick filaments that gave them features very similar to the longitudinal muscle cells. Those cells were clearly restricted to the 2～3 cell layers at the periphery of the circular muscle bundle apposed to the mucosal layer. Nexuses could not be found between these cells. The aspect of these cells including the lack of nexuses coincides with the special cells reported by Gabella[14] in the circular layer of the ileum of the guinea pig and of other animals. These cells are not illustrated here.

## Discussion

The longitudinal and circular muscle cells of the guinea pig stomach proved to be different from each other in structure as well as in contractile response. Although most of the structural differences were quantitative ones, the longitudinal muscle cells contained higher concentrations of SR, mitochondria and microtubules, and, as a result, contained a relatively small mass of myofilaments. All these differences gave the longitudinal muscle cell an appearance different to that of the circular muscle cell.

The circular muscle showed greater contraction, and quicker relaxation than the longitudinal muscle, when stimulated with K-depolarizing solution, or with single or repetitive electrical stimuli (present observation ; Kuriyama *et al.*[1]). A possible explanation for the quicker relaxation in the circular muscle strip might be related to the calcium-sequestering function of SR, as has been shown in striated muscle[15]. Unexpectedly, we observed that there was more SR in the longitudinal muscle cells than in the circular muscle cells. Therefore, the amount of SR does not seem to be related directly to the control of cytoplasmic calcium concentration in smooth muscle. Other possible mechanism to lower the cytoplasmic free calcium concentration to cause relaxation are, a) Na-Ca exchange[16], and b) Ca extrusion at plasma-membrane supported by Ca-activated ATPase[17]. However, we shall not be able to obtain any information about these mechanisms through a morphological study.

The other structural difference which seemed to be important related to the contractile filaments. As the myofilaments (thick and thin filaments) are regarded as a force-generating apparatus, differences between these filaments should be related to differences in patterns of contraction. We found thick filament to be more sparsely distributed and less numerous in the longitudinal than in the circular muscle cell. This fact was further confirmed with experiments using other buffer solutions for electron microscopy. The use of lower pH fixative (pH 6.6), which has been reported to prevent myosin extraction from smooth muscle[9,18] slightly increased the density of thick filaments (Table 2), perhaps by improving the contrast of thick filaments, but the density in circular muscle was still twice that of the longitudinal muscle. These observations suggest differences in myosin content, or otherwise in myosin aggregation between the two muscle tissues.

Nexuses were consistently found in the circular muscle layer, but could not be found in the longitudinal muscle layer under our experimental conditions. This fact was also reported in duodenum[19], ileum[20] and caecum[21]. The present data further confirmed the prediction that the nexus is a characteristic structure of circular muscle of the gut. The nexus has been supposed to be a morphological correlate of electrical coupling between smooth muscle cells[22]. In this connection, however, Kuriyama *et al.*[23] reported nearly the same length constant in the longitudinal and circular smooth muscle (1.2 to 1.5) of the guinea pig stomach. Electrical coupling might therefore occur in the longitudinal muscle in the absence of typical nexuses. A specific function of the nexuses localized in the circular layer is not clear.

The significance of every structural difference described above for their contractile responses remains to be clarified. The strength of contraction of these two muscle layers (e. g. for maximum developing force, maximum shortening velocity) is now under experiment, in order to assess the significance of thick filaments for the contraction of smooth muscle.

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# モルモット胃の縦走筋と輪走筋細胞の構造的差違

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## 要 約

収縮パターンや，薬物反応に顕著な差を示すモルモット胃の縦走筋と輪走筋細胞の微細構造を観察し，比較検討した．胃体部筋層の外部1/3が縦走筋層で，約20個の筋細胞列で構成されており，その細胞間隙の面積率は約12.1%であった．輪走筋層は筋細胞が密につまっており，その細胞間隙は約4.4%であった．輪走筋層にのみNexus構造が観察された．筋細胞の単位断面積当りに出現するミトコンドリア，微小管はいずれも縦走筋細胞により多く，また筋小胞体によってしめられる面積率も，縦走筋では4.7%で，輪走筋の約2倍であった．残りの細胞質は収縮性フイラメントによってしめられており，thick, thin filamentが観察された．しかし縦走筋細胞ではthick filamentが輪走筋のものにくらべより細く，分布密度も，輪走筋では0.5 $\mu m^2$ 当り50本に対し，縦走筋では約25本であった．この事実は，この両筋細胞では，収縮装置の形態にも差があることを示唆している．